

PLACENTATION IN THE LABORATORY MOUSE

by

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INTRODUCTION AND LITERATURE REVIEW

With the phenomenal expansion of the medical sciences the laboratory mouse has become one of the standard experimental animals. White mice are used by the millions in cancer research and in the proving of pharmaceuticals for use on humans. A large part of this pharmaceutical development has revolved around the advent of hormonal control of the estrous cycle as therapy for sterility or as a birth control measure.

Before the pathologies brought about by the exogenous substances being tested can be recognized and understood a thorough knowledge of the total developmental processes must be at hand. Placentation is an important part of gestation. The last comprehensive study on the mouse was done in 1940 (Snell, et al.) before the rapid expansion of the field. Placentation was not treated as a separate subject in the paper but mentioned in passing during the description of the embryology of the mouse. Most of the information on placentation of the mouse is expressed secondarily in findings on the development of giant cells and placental transfer of compounds and ions. Some work has been done on the development, morphology and physiology of the albino rat placenta (Huber, 1915; Everett, 1935; Bridgman, 1948; Boe, 1950), and mouse placentation has been homologized with rat placentation.

Ovulation in the white mouse is spontaneous, occurring independent of coitus (Long and Mark, 1911). It occurs during estrus at about the time vaginal smears show complete cornification. Ovulation time varies and the vaginal smear technique was found to be unsatisfactory in determining onset of heat in the guinea pig and rat (Young, Boling and Blandau, 1941). Most copulations occur between 10 PM and 1 AM. A few occur later. In the white mouse, mating and ovulation are closely associated, mating usually preceding

ovulation by up to three hours. Ovulation occurs between midnight and 3 AM, occasionally as late as 4:40 AM (Snell et al., 1940).

Ova are in the metaphase stage of the second maturation division at the time of ovulation, the first maturation division having been completed while the ova were still within the ovary (Coe, 1907). The first polar body may or may not accompany the ovum after ovulation. Fertilization stimulates the ovum to complete the formation of the second polar body (Kirkham, 1907).

Cleavage begins 2½ hours post coitum, the first division taking about ten minutes for completion. The second division follows the first by 12 hours, followed 10 hours later by the third division. The fourth division occurs 8 hours later, at about 5½ hours post coitum, and results in a 16-cell morulla. It is in this stage that the embryos, clustered together, enter the uterus (Rugh, 1964).

In the uterus the morulla becomes a "blastocyst" by development of a central cavity. The blastocysts become equidistantly spaced along the length of the uterus and settle into crypts in the antimesometrial surface of the endometrium. The zona pellucida is shed at the eight cell stage, before the embryo settles into the crypt (Robinson, 1904; Wilson, 1963; Rugh, 1964).

The embryo is now a hollow ball. The thin wall is a single cell thick, except at the mesometrial pole where there is a mass, several cells thick, the inner cell mass (Jenkinson, 1900; Rugh, 1964). Jenkinson (1900) stated that the trophoderm is continuous over the top of the inner cell mass but Huber (1915) disagreed. Robinson (1904) stated that the inner cell mass differentiates three types of cells: internal trophoblast (extraembryonic ectoderm), amnio-embryonic ectoderm and endoderm.

The blastocyst comes in contact with the uterine epithelium and giant

cells appear in the trophoderm at the point of contact. Rugh (1964) saw giant cells at $4\frac{1}{2}$ days, Alden (1948) at $5\frac{1}{2}$ days and Bridgman (1948) distinguished them at 6 days. If the blastocyst settles to the bottom of the crypt, the first giant cells make their appearance in the abembryonic trophoderm (Bridgman, 1948; Rugh, 1964). If first contact is made equatorially, the trophoderm cells at this point enlarge and form primary trophoblastic giant cells. The giant cells formed in the ventral trophoderm extend themselves to reach the uterine epithelium (Alden, 1948).

Processes from the giant cells may extend to the base of the uterine epithelium and assist in its breakdown by forcing the epithelium into the uterine lumen (Bridgman, 1948). Alden (1948) stated that the abembryonic giant cells possibly may form a primitive syncytium which moves along the basement membrane under the epithelium, prying portions loose. In the blind ends of the crypts the uterine epithelium proliferates to fill the lumen, then is absorbed or disappears (Robinson, 1904; Alden, 1948).

At this time in the embedding process, a reaction by the maternal tissue is elicited, the "decidual reaction." Endometrial growth and swelling soon closes the connection between the crypt and the uterine lumen and in time the entire uterine lumen dorsal to the embryo. In this manner the embryo comes to lie within the mucosa of the uterus. This is not an active penetration (Alden, 1948; Rugh, 1964).

During the 7th day of gestation, smaller secondary giant cells appear at the point where the basal edges of the forming ectoplacental cone come in contact with the uterine epithelium dorsal to the embryo (Bridgman, 1948; Alden, 1948). The ectoplacental cone forms from a thickening of Rauber's layer (Jenkinson, 1900), and is conical in shape with its apex extending

toward the uterine lumen and its base resting upon the extraembryonic ectoderm of the inner cell mass. The base of the cone, at its edges, is continuous with the trophoblast. The uterine epithelium of the crypt between the embryo and the uterine lumen is destroyed cell by cell as the expanding cone advances (Robinson, 1904; Alden, 1948). Giant cells migrate ventrally from the ectoplacental cone forming a loose network of giant cells surrounding the trophoblastic vesicle. Protoplasmic processes of the giant cells span blood spaces filled with circulating maternal blood. The trophoblast becomes blended with maternal tissue (Robinson, 1904; Alden, 1948; Bridgman, 1948; Rugh, 1964). Rugh (1964) considered that the giant cells aid in securing the trophoblast to maternal tissue.

At about six days a structure peculiar to rodents appears, "Reichert's membrane." This is a non-cellular, tough, elastic membrane between the distal endoderm and the trophoblast (Rugh, 1964). The blood channels spanned by the trophoblastic giant cells bring circulating maternal blood in contact with Reichert's membrane. Nutrients pass through Reichert's membrane and are absorbed by the distal endoderm and pass into the yolk sac cavity, from which the nutrients are readily available to the embryo (Robinson, 1904; Rugh, 1964). The endometrium around the ectoplacental cone becomes filled with maternal blood channels that bathe the ectoplacental cone with blood (Bridgman, 1948).

During the 7th day, extraembryonic mesoderm begins to grow from the posterior end of the embryo and expands into the exocoel. This is the "allantois", which lacks an endodermal cavity at all times during its development. The allantois expands into and eliminates the exocoel (Robinson, 1904; Rugh, 1964). Above the exocoel forming its dorsal wall is the "lamina", separated from the ectoplacental cone by the ectoplacental cavity. As the allantois expands it contacts and fuses with the lamina or "chorion" by late day nine

forcing a concavity in the base of the cone (Robinson, 1904; Everett, 1935; Bridgman, 1948).

With the fusion of the allantois with the chorion, the ectoplacental cone increases in size rapidly. The maternal blood spaces within the cone enlarge (Robinson, 1904) and are continuous with those in the surrounding decidua (Bridgman, 1948).

Villus projections containing fetal (allantoic) blood vessels invade the lamina and ectoplacental cone (Robinson, 1904; Everett, 1935) forming a "labyrinth" (Bridgman, 1948). The ectoplacental cone is a trophoblastic syncytium (Jenkinson, 1900; Robinson, 1904), from which fetal plasmodesia invade the maternal vessels, destroying the swollen maternal endothelium (Bridgman, 1948). The choriocallantoic villi continue to erode the cone material up to the cytotrophoblastic cap that forms the roof of the labyrinth (Everett, 1935).

The cone is now the "junctional zone" with those cone cells that have remained unchanged among the syncytial strands becoming the "glycogen cells" (Bridgman, 1948). The "central zone" lies to the sides of the embryonic vesicle, peripheral to the developing placental pad. The mass of spindle-shaped giant cells comprise the spongy tissue that lies against Reichert's membrane and surrounds maternal blood channels (Everett, 1935).

Mitotic activity decreases in the cone during the 9th day and increases in the lamina. During the 12th day mitotic activity increases in the cone but is rare in the labyrinth. By the 14th day, when all the trophoderm cells in the labyrinth are syncytial, there is no mitosis in the region, and there is mitosis in the junctional zone only along the circumference of the pad. No mitotic stages are found in the fetal placenta after the 16th day

nor in the surrounding decidua after day 11 (Bridgman, 1948).

The greater portion of the junctional zone is composed of syncytium by the 17th day. Fetal and maternal glycogen cells become intermingled in the central region of the placenta. At parturition the maternal glycogen cells collapse, separating the fetus and vesicle from the uterus (Bridgman, 1948).

Mossman (1937) described the placenta of Mus musculus as hemo-endothelial though Bridgman (1948) stated that syncytial trophoblast is found between maternal blood spaces and fetal blood vessels in the rat. Wislocki (1955) studied the placenta of the rat with the electron microscope and found three layers of tissue between maternal blood and fetal endothelium. He suggested an overlapping of the trophodermal cells as a possible explanation of the multiple layers. Enders (1965) described the definitive placenta of the white mouse as hemo-trichorial with three layers of trophoblast between the allantoic endothelium and the maternal blood spaces. Enders stated that Mossman had interpreted the three layers as (1) a layer of trophodermal giant cells in contact with maternal blood spaces, (2) a syncytial layer originating from the ectoplacental cone cells, and (3) a layer from the chorionic ectoderm also probably syncytial.

Poe (1950), by a series of India ink injections and corrosion of rat placentae, found the maternal blood supply to the placenta enters centrally and spreads throughout the labyrinth of maternal blood spaces. At the venous end, blood collects peripherally in the pad. On the embryonic side allantoic arteries enter the chorioallantoic villi and ramify throughout the labyrinth coming in close proximity to the maternal blood spaces.

METHODS AND MATERIALS

A laboratory-maintained strain of Swiss White mice was used for this study. Two females were placed with a male in an 8" x 8" x 11½" wire cage. The mice were fed a proven pelleted laboratory feed. Water was supplied ad libitum. The lights in the laboratory were on a time switch for 15 hours of light a day from 5 AM to 8 PM.

Each female was checked daily between 8:00 and 8:30 AM. When a copulation plug was observed, that female was placed in a separate cage, thus eliminating later insemination in case pregnancy did not result from the first copulation.

Age of the embryos was counted in hours from 2 AM, the morning the plug was observed to time of sacrifice. Animals were sacrificed to provide a series at six hour intervals through the first half of the 11th day of gestation and at 24 hour intervals day 12 through day 18. Attempts to obtain stages for days 19 and 20 were unsuccessful because parturition occurred prior to the time of sacrifice. Fifty-two sets of closely spaced, accurately aged mouse embryos were collected for study.

At the time of sacrifice the pregnant females were euthanized with chloroform. The uteri and ovaries were dissected out, spread on a wax bottom dish and covered with Bouin's fixative. The uteri were left stretched for 15 to 20 minutes to minimize muscular contraction then transferred to a small jar, covered with Bouin's fluid and allowed to fix for at least 24 hours.

To secure the early embryos, the oviducts were serially sectioned. In stages later than 80 hours, but with no visible swellings, part of one

uterine horn was sectioned serially. After 120 hours the decidual swellings were trimmed out for processing.

Four animals were sacrificed at stages with uterine embryos prior to the visible swelling stage. The reproductive tracts were removed, stretched and fixed in formolacetic acid (10% formalin, 5% glacial acetic acid) for 24 hours, then dehydrated with isopropyl alcohol to 95%. They were transferred to a 50-50 solution of absolute alcohol and benzylbenzoate for 24 hours and then to benzylbenzoate until cleared (Orsini, 1962). Embryos in the oviducts were not detectable but the technique was excellent for locating decidual sites that were not visible to the eye. Sites of decidual reaction so determined were excised for sectioning.

All specimens for sectioning were dehydrated with isopropyl alcohol, infiltrated and embedded in paraffin. They were sectioned at eight microns and stained with one of three stains: hematoxylin-acid fuchsin-orange G, hematoxylin-Mallory's triple, or periodic-acid-Schiff. Picolyte in xylol was used as a mounting medium.

At least one transverse and one longitudinal serial was made for each stage, and in many cases, each of two or three embryonic swellings from one uterus were sectioned transversely or longitudinally.

Serial sections were studied by projection and microscope. Composite drawings were made where necessary to establish an understanding of relationships. Photomicrographs were taken at appropriate magnifications with an Exacta camera on an extension bellows.

OBSERVATIONS

ATTACHMENT, DESTRUCTION OF THE UTERINE EPITHELIUM. Days 4 & 5.

During days four and five no gross changes are obvious in the uterus. No swellings are visible at this time and the uterus is about 1.5 to 2 mm in diameter.

The Embryo. When the embryos enter the uterus, approximately 80 hours post ovulation, they are early trophocysts with an eccentrically located trophocoel (Fig. 1). By some unexplained means the embryos space themselves relatively equidistant along the length of the uterus. The embryos then settle into "crypts", which are tube-like projections that extend ventrally off the uterine lumen. The trophocoel enlarges, stretching the trophoderm cells to flattened plates with long, oval nuclei. The inner cell mass is oriented toward the mesometrium with the flattened trophoderm continuous over the inner cell mass as Rauber's layer.

At day four the trophocyst is about 230 microns from embryonic to abembryonic pole. Rauber's layer is distinct as a layer of flattened nuclei oriented parallel to the surface of the inner cell mass. A distinct layer of endodermal cells has delaminated along the ventral surface of the inner cell mass. These endoderm cells are smaller and darker staining, with nuclei about half as large as those of the epiblast cells (Fig. 2). During day five there is an increase in the number of cells within the inner cell mass causing the embryonic mass to expand downward into the yolk sac cavity.

The Endometrium. The uterine lumen is eccentric toward the mesometrium and runs an erratic path the length of the horn. The lumen is lined with

epithelium that varies from columnar to pseudostratified columnar around the uterine lumen proper and is regularly low columnar in the area of the junction of crypt lumen and uterine lumen. In the area of the crypt occupied by the trophocyst, the epithelium is further reduced to an even cuboidal layer. Ventral to the trophocyst the crypt epithelium is fifty percent higher than normal in places and pycnotic nuclei are found free in the crypt lumen.

Surrounding the epithelium of the crypt is a thin, densely packed layer of endometrial cells with dark staining cytoplasm and enlarged, plump nuclei. This is the beginning of the "decidual" reaction to the presence of the embryos in the uterine crypts. Beyond this is an extensive area of loosely organized stratum spongiosum. Uterine glands penetrate from the uterine epithelium to the surrounding circular muscle peripherally and antimesometrially. Mesometrially there is only a thin band of endometrium between the uterine lumen and the circular muscle. Surrounding the circular muscle layer is a thick, convoluted layer of longitudinal muscle.

Blood vessels enter through the mesometrium and branch between the circular muscle and longitudinal muscle, penetrate the circular muscle layer and ramify throughout the endometrium. There is an extensive proliferation of vasofactive cells toward the crypt lumen.

Embryonic - Endometrial Relationships. First direct contact between maternal and fetal tissues occurs at about 90 hours post ovulation and in the mouse usually occurs equatorially between the peripheral trophoderm and the adjacent uterine epithelium. Trophoderm cells, at the point of contact with uterine epithelium, lose their flattened appearance, swell somewhat and their nuclei become round and vesicular. They send out pseudopodia that wedge

between uterine epithelial cells forcing gaps into which the nuclei move (Fig. 3). These trophoderm cells are probably the "primary invasive cells" referred to by Wilson (1963). Once the epithelium is breached its destruction progresses by both cytolysis and phagocytosis.

During the fourth and fifth days of gestation the embryo accomplishes the breakdown of the uterine epithelium in the immediate area. By the end of the fifth day only the epithelium lateral to the embryo has been destroyed. There is no destruction beyond the area of contact although the crypt epithelium ventral to the embryo shows early stages of necrosis.

With the uterine epithelium gone the embryonic trophoderm is in contact with the endometrial stratum compactum. The decidual reaction increases to include a band about five to six cell layers and the uterine gland tissue has disappeared within the reacting region. By the end of the fifth day the decidual reaction has caused no discernable swelling of the uterus. If uteri of 100 to 120 hours are fixed in formolacetic acid and cleared with benzyl-benzoate, the decidual sites may be seen with the aid of a dissecting microscope.

THE ECTOPLACENTAL CONE STAGE. Days 6, 7 & 8.

The pregnant uterus is approximately 2 mm in diameter as day six begins. Soon swellings become evident, each swelling denoting the location of a developing embryo. The uterine swellings are 3 mm thick by the end of the eighth day.

The Embryo. At day six the embryonic mass has enlarged, pushing down into and almost filling the yolk sac cavity mainly by an increase of the inner cell mass (Fig. 4). The embryonic mass is covered ventrally and

laterally by endoderm which reflects back as the distal endoderm. During the sixth day the distal endoderm consists of a few cells in a discontinuous sheet covering the inner surface of the peripheral trophoderm mesometrially. By mid day seven endodermal cells have proliferated and expanded forming a continuous lining of the entire peripheral trophoderm (Fig. 5).

Differential growth of the ectoderm of the inner cell mass during day six causes the embryonic mass to assume a figure eight shape (Fig. 7). The dorsal or mesometrial sphere of cells is extraembryonic ectoderm, associated with extraembryonic endoderm. The ventral or antimesometrial sphere is composed of embryonic ectoderm and endoderm. The endoderm varies from a high cuboidal layer associated with the extraembryonic ectoderm to a low cuboidal to squamous layer over the embryonic ectoderm.

Cells in the center of the embryonic mass become necrotic early in day six and within a few hours break down, forming the "proamniotic" cavity (Fig. 6). By the middle of the sixth day the central cells of the extraembryonic portion of the embryonic mass have broken down forming a continuous cavity within the mass.

Mesoderm appears during the seventh day, at the posterior end of the embryo in the area of the primitive streak, and spreads anteriorly between the embryonic ectoderm and endoderm and posteriorly between extraembryonic ectoderm and endoderm. The extraembryonic mesoderm soon splits forming somatic and splanchnic mesoderm separated by extraembryonic coelom. The somatopleure reflects anteriorly over the embryo (Fig. 10) as the posterior amniotic fold. By late day seven the mesoderm has reached the anterior portion of the embryo and a similar but much smaller coelomic cavity is formed resulting in a relatively small anterior amniotic fold. By the end of day seven the two

amniotic folds have met and fused thus forming amnion and serosa. The cavity between the serosa and amnion is the extraembryonic coelom and is bounded dorsally by the somatic mesoderm of the serosa, ventrally by the somatic mesoderm of the amnion and laterally by the splanchnic mesoderm of the yolk sac (Fig. 11).

The formation of the extraembryonic coelom and membranes divides the single cavity within the embryonic mass into three cavities. The ventral cavity within the embryonic ectoderm is the amniotic cavity and is separated, by amnion, from the extraembryonic coelom which in turn is separated from the dorsal ectoplacental cavity by the serosa. The ectoplacental cavity is bounded dorsally by the base of the ectoplacental cone (Fig. 11).

Late in day eight, nodules of tissue form within the splanchnic mesoderm of the yolk sac. These nodules are blood islands consisting of a central mass of blood forming cells surrounded by a thin squamous endothelium.

Ectoplacental Cone. The ectoplacental cone appears during day six as a proliferation of Rauber's layer. In early day six it is two to three cells thick oriented across the top of the embryonic mass. By mid day six the ectoplacental cone has increased in size and is roughly triangular in section approximately 100 μ across the base and 100 μ high. The apex of the cone is directed dorsally toward the uterine lumen. The lateral edges of the base of the cone are continuous with the peripheral trophoderm (Fig. 6).

The ectoplacental cone differentiates two structurally different areas. The inner portion is a solid mass of cells still oriented across the top of the ectoderm of the embryonic mass (Fig. 8). The apical half of the cone forms intercellular vesicles which coalesce into a network of channels by the end of day six (Fig. 11). By day seven the channels cover the entire surface

of the cone that is in contact with the endometrium.

Peripheral Trophoderm. Peripherally the trophoderm, in contact with the endometrium, is one or two cells thick during day six. The trophodermal cells are still flattened with only an occasional large round cell. The trophodermal cells along the ventral pole have enlarged enormously and are truly giant cells. These giant cells are instrumental in the removal of the epithelium in the blind end of the uterine crypt. The peripheral trophoderm cells during the seventh day are less flattened and comprise a layer two or three cells thick. They aid in the removal of the crypt epithelium and the eventual destruction and elimination of the decidua.

Reichert's membrane appears during day seven as a non-cellular, tough, elastic, homogenous membrane formed between the distal endoderm and the peripheral trophoderm. The peripheral trophoderm cells form a loose meshwork connected to each other and to Reichert's membrane by cytoplasmic processes. By the end of the seventh day, the network of intercellular channels between the trophoderm cells surrounds the embryonic vesicle and is continuous with the network on the surface of the ectoplacental cone.

Endometrium. During day six vacuoles appear between the endometrial cells forming a frothy band about eight to ten cells thick surrounding the embryonic vesicle. By the end of the sixth day, vacuolization has spread mesometrially surrounding the uterine crypt neck, almost to the uterine lumen. The decidual reaction spreads peripherally until by the end of day eight only a thin band of stratum spongiosum remains unmodified. The tips of the uterine glands remain in fairly normal condition, containing PAS+ material, until by late day seven they too become nonfunctional, disorganized clumps of necrotic tissue and by day eight have practically disappeared.

An increase in the capillary bed is apparent at day six. Vasofactive cells are evident throughout the endometrium and many of the capillaries have begun to enlarge into sinuses. By day seven the sinus network has become extensive, most evident in the mesometrial half of the endometrium, appearing to fan out from the dorsal end of the embryonic vesicle at a 45 degree angle dorso-laterally. Sinus development is less extreme peripherally and ventrally. The peripheral sinus network is extensive up to the distal edge of the band of intercellular vacuolization. Few capillaries penetrate the vacuolated area although the area is vascularized with an occasional capillary penetrating to the *membrana propria*.

Endometrial - Embryo Relationships. The trophodermal cells in contact with the endometrium breach the *membrana propria* during day six and begin the destruction of the endometrium. By the middle of the seventh day the entire area of intercellular vacuolization has been eliminated and the extensive vascular sinus region has been reached. Continued destruction of the endometrium ruptures these blood sinuses and maternal blood flows through the mesh-work formed by the peripheral trophoderm cells. Mesometrially the ectoplacental cone cells rupture the large mesometrial blood sinuses and maternal blood flowing from these breaches enters the network within the ectoderm of the cone. By the end of the seventh day the entire cone, with the exception of the basal portion connected to the embryonic mass, is vascularized. There is now a continuous vascular meshwork with circulating maternal blood surrounding the embryonic vesicle (Fig. 11).

As the decidual reaction spreads through the endometrium, it increases from about 600 microns in thickness on day six to about 1 mm during day eight. The embryonic vesicle increases in diameter from 70 μ on day six to about 600

on day eight. The enlargement of the uterine swelling from 2 mm to 3 mm during this period is a reflection, primarily, of an increase in the total amount of endometrial tissue caused by the hypertrophy occurring in the decidual cells. The embryonic vesicle increases about eight fold during the 72 hours from day six through day eight while there is not quite a doubling of the endometrial tissue. By the end of day eight the endometrium has reached its maximum thickness.

FORMATION OF THE CHORIONIC VILLI. Days 9 & 10.

Embryo. The blood islands in the yolk sac remain solid throughout the ninth day with the inner cells undergoing development into blood cells. There is some release of newly formed blood cells into the embryonic vessels by the middle of the ninth day and occasionally blood cells are found in the posterior portions of the dorsal aorta. Circulation, as such, does not occur until early in the tenth day at which time all the major vessels and the heart contain quantities of blood.

Late in day eight a mass of mesoderm appears at the posterior end of the embryo just dorsal to the tail fold. This mesodermal mass is the body stalk and lacks an endodermal lined cavity throughout its development. During early day nine the body stalk enlarges, pushing out into the extraembryonic coelom.

Limiting the extraembryonic coelom dorsally is the serosa, with somatic mesoderm exposed to the coelom. The ectoderm of the serosa is exposed to the ectoplacental cavity which is limited dorsally by the base of the ectoplacental cone. The basal two or three cell layers of the cone are oriented across the top of the ectoplacental cavity (Fig. 13).

The body stalk contacts the serosa early in day nine and within a few

hours has spread over the entire surface of the serosa. Continued growth of the body stalk forces the serosa dorsally against the base of the cone obliterating the ectoplacental cavity. The proximal half of the body stalk is a solid mass of mesenchymal tissue. The bulbous, distal portion is a loosely organized mesenchymal mass. The allantoic arteries develop from the dorsal aorta during the middle of the ninth day and penetrate the solid proximal portion of the body stalk where they continue dorsally through the peripheral portion of the distal body stalk.

Villous Formation. The serosa is forced against the ectoplacental cone base by the body stalk and fuses with it becoming indistinguishable from the cone ectoderm. The serosal ectodermal cells lose their low cuboidal or squamous appearance becoming a columnar layer across the base of the cone (Fig. 14). It is difficult to positively identify a distinct layer of mesoderm as serosal mesoderm. By the middle of the ninth day the ventral surface of the serosa appears uneven. Within each of these indentations is an allantoic capillary (Fig. 15). With the vascularization by the allantoic blood vessels, the serosa becomes a true chorion. The capillaries penetrate the mesoderm of the body stalk and serosa to come in direct contact with the serosal ectoderm. In some cases mesoderm was observed to line the villi in a position between the capillary and the chorionic ectoderm.

The chorionic villi form as the allantoic blood vessels push dorsally, accompanied by rearrangement of the chorionic ectoderm cells into a columnar epithelium investing each villus. Many mitotic figures occur in the chorionic ectoderm.

During day nine the developing villi have caused only a slight unevenness in the ventral surface of the chorion (Figs. 14, 15 & 27) but by the

early part of the tenth day short, straight chorionic villi 40-60 μ in length have pushed into the central portion of the chorion (Fig. 16). Such villi develop over all the ventral surface of the chorion by the end of day ten. Embryonic blood appears in the allantoic blood vessels of the body stalk and the capillaries of the chorionic villi by the middle of the tenth day.

The chorionic villi become tortuous and branched during the last half of day ten. The chorionic ectoderm is stretched into a thin layer separating fetal capillaries from maternal blood as the growing chorionic villi attain a length of about 250 μ pushing dorsally into and across maternal blood spaces within the ectoplacental cone (Figs. 17 & 28). As the chorionic villi push into the base of the cone, each villus is invested with a layer of cone cells.

Peripheral Trophoderm - Endometrium Relationships. The uterine swellings increase in size from about 3 mm at the end of day eight to about 5.5 mm at the end of day ten. This rapid increase in size is due to the increase in size of the embryonic vesicle as the destruction of the peripheral and ventral endometrium by the embryonic trophoderm has exceeded the rate of increase due to the decidual reaction at the beginning of the ninth day. A "line of penetration" has become evident as a line of pycnotic nuclei, within the endometrium, just distal to the peripheral trophoderm cells, marking the limit of the destructive influences of the trophoderm. The trophodermal cells phagocytize the debris from the degenerating endometrial cells. Trophodermal cells attack the adjacent endometrium directly, with the advance maintained on a steady front, not up the maternal blood sinuses as has been described for the rat (Bridgman, 1948). The endometrium decreases in thickness from about 1 mm at the end of the eighth day to about 400 μ by the end of the tenth day. On

the anterior and posterior ends of the embryonic swelling there remains only a thin layer of endometrium, 100 to 150 μ in thickness, between the trophoderm and the old uterine lumen.

During the latter half of day ten the metrial gland appears mesometrially. The first cells are found within the circular muscle layer just ventral to the mesometrium. The cells are rounded, approximately 20-25 μ in diameter, with nuclei about 10 μ in diameter. The metrial tissue usually appears more densely packed than the adjacent endometrium and therefore stains a little darker.

A rudimentary but functional placenta has been established by the end of the tenth day. Observation of the tissues separating fetal blood from maternal blood reveals an endothelium comprising the fetal blood vessel, a layer of chorionic ectoderm and a layer or layers of ectoplacental cone cells. In the basal portions of the cone that are thoroughly penetrated by chorionic villi, the chorionic ectoderm cells that invest the villi are drawn quite thin. It is difficult to discern the exact nature of the investing layers at this point. Mesometrially several cone cells may separate maternal blood from the allantoic capillaries.

COMPLETION OF THE PLACENTA. Days 11 through 14.

Embryo. During this period the embryo grows rapidly, the crown-rump measurement increases from 4.3 mm on day 11 to about 9.5 mm on day 14. The 11 day fetus is sharply C shaped with limb buds present. The maxillary processes have not fused completely in the midline. By 14 days the fetus is rolled into a ball, hair and vibrissae papillae are present, the digits are distinct. The fetus is definitely mouse-like.

The uterine swellings increase from about 6 mm on day 11 to 10 mm on day 14. The 14 day fetus occupies almost the entire space within the embryonic vesicle. The amnion is drawn tightly over the fetus eliminating an amniotic cavity as such.

The Placenta. Three distinct areas have become evident by the end of the tenth day (Fig. 28). The basal portion of the placental pad is referred to as the "labyrinth" and is that area of the ectoplacental cone that has been invaded by the chorionic villi. The villi have pushed into the maternal blood channels within the cone so maternal blood and fetal blood are in close proximity.

The placenta undergoes a rapid growth from about 450μ thick by 3.1 mm wide on the 11th day to about 1.3 mm thick by 7 mm wide on day 14. The mesometrial endometrium limits any major increase in placental pad size in that direction. Therefore the major increase in size occurs ventrally and laterally. The peripheral trophoderm and distal endoderm, separated by Reichert's membrane, remain in position against the peripheral endometrium. As the placental pad increases in thickness, Reichert's membrane is carried ventrally with it resulting in a fold in the membrane around the circumference of the pad (Figs. 20 and 26).

The labyrinth region increases in width from 3 mm to 6 mm from day 11 through day 14. The chorionic villi have increased in length from 300μ to about 1 mm during the same period. The first villous projections of day ten become the main villous trunks of the definitive placenta. As each main villus increases in length and complexity it pushes into the cone tissue mesometrially. The ectodermal cells of this "laminar" region, are incorporated into the covering of each branch, separating fetal capillaries from

maternal blood. The penetration of each villus is more rapid mesometrially than branching is laterally, leaving laminar septae between the main villous trunks. These septae, usually containing maternal blood channels, are found in the 14 day placenta and are of varying thickness.

The second distinct area, the laminar region of the placenta, decreases from about 170 μ at day 11 to a variable layer only a few cells thick by 14 days. Between this laminar region and the dorsal endometrium is a loose layer of ectoplacental cone cells surrounding maternal blood channels which connect with those of the labyrinth region by way of channels within and through the laminar ectoderm. This dorsal trophodermal meshwork, the third area, is still quite extensive (250 μ thick) day 14 and is in contact with the dorsal endometrium.

Embryonic - Endometrial Relationships. As the placenta and embryonic vesicle enlarge they do so at the expense of the maternal tissue. Mesometrially and laterally to the pad the giant cells and trophodermal cells comprising the maternal blood channels continue their destruction of the endometrium between the fetal tissue and the circular muscle layer, reducing it from 1.5 mm in thickness on day 11 to about 200 μ by the end of day 14.

On day 11 the endometrium lateral to the embryonic vesicle is about 170-175 μ thick and 200-250 μ ventrally. Anteriorly and posteriorly, where the embryonic vesicle has pushed out into the uterine lumen, there remains a layer of endometrium only five or six cells thick between the trophoderm and the uterine lumen. These remaining cells are drawn out spindle shaped by the increase in size of the embryonic vesicle.

All the endometrium surrounding the vesicle laterally and ventrally is destroyed during day 12. After the endometrium is eliminated, the peripheral

trophoderm disappears (Fig. 25). By day 14 the only remnant of the encapsulating endometrium is a short degenerating strip just lateral to the placental pad extending for about 1.5 mm between Reichert's membrane and the uterine epithelium. Reichert's membrane and the distal endoderm are intact until the latter part of day 14 when they rupture, drawing up into a crumpled mass around the periphery of the placental pad. With the rupture of Reichert's membrane, the embryonic vesicle is in the uterine lumen. The endodermal layer of the yolk sac covers the outer surface of the vesicle and is pressed against the uterine epithelium by pressure from within the vesicle.

Metrial Gland. On day 11 the metrial gland has developed a distinct integrity of shape. It has become a disc 400 μ thick by 3 mm wide. By day 14 it has increased to a little over 1 mm thick by 4 mm wide (Fig. 19-20). The gland develops within the circular muscle layer, widely separating the layer. Muscle fibers run across the substance of the gland. A large number of the metrial gland cells contain PAS positive material.

THE DEFINITIVE PLACENTA. Days 15 through parturition at 19 days.

The uterine swellings increase in thickness from 10 mm on day 15 to 14 mm at parturition. During the definitive stages it becomes possible to measure the length of the swelling with some accuracy. Before the 15th day the swellings in a uterine horn are so close together that it is difficult to delineate the anterior aspect of one swelling from the posterior aspect of another. The length of the swellings increases from about 9 mm on day 15 to 19.5 mm at parturition.

With the increase in size, the uterine wall is stretched thin. The

fetus is visible through the wall of the uterus during this time. The placental pad becomes evident, externally, as a cap mesometrially within the uterine swelling about 1 to 2 mm thick by 7 or 8 mm wide.

The placenta is complete by the 15th day and there are no major changes in size or structure after this time (Fig. 21). The placental pad is 1.3 mm thick by 7 mm wide at the beginning of the 15th day and only increases to about 2 mm by 7 mm at parturition. By parturition continued growth of the chorionic villi has increased the thickness of the labyrinth region from 1 mm on day 15 to 1.5 mm.

The labyrinth region comprises the majority of the definitive placental pad. Some laminar ectoderm is still present along the mesometrial surface of the labyrinth and as septae within the labyrinth between main villous branches. The laminar tissue blends so well with the outermost layer of loose trophoderm which surrounds maternal blood channels that an interface is not distinct. At the periphery of the term placental pad occasional trophodermal giant cells are still present (Fig. 22).

At parturition the tissue separating the fetal blood from maternal blood is composed of the endothelial lining of the allantoic capillaries and a layer of trophoderm. With the light microscope it is impossible to distinguish the exact nature of the intervening trophoderm. The definitive placenta of Mus musculus is hemochorial (Fig. 32).

Metrial Gland. During the period from day 15 through parturition the metrial gland does not maintain its distinctive shape. Several large blood vessels pass through and supply the area of the metrial gland, distorting the shape of the gland. By day 18 the general dimensions have decreased to approximately 600 μ thick by 3.5 mm wide. At parturition the metrial gland, as such, is no longer discernable (Fig. 21 and 22).

DISCUSSION

Little work has been done on placentation in the mouse although several excellent papers describe the early development of the mouse (Coe, 1907; Kirkham, 1907; and Snell et al., 1940) and electron microscope studies have been reported on the nature of the placental barrier in the definitive placenta (Kirby and Bradbury, 1965; Enders, 1965). None of these reports included development of the placenta.

Placentation in the rat has been explored to a greater extent (Huber, 1915; Everett, 1935; Alden, 1948; Bridgman, 1948; Wislocki and Dempsey, 1955). Kirby and Bradbury (1965) and Enders (1965) stated that the placentas of the mouse and rat were identical. In the following discussion, some comparisons will be made to show that placentation in the mouse is not identical with that described for the rat.

The term blastocyst has been applied to the mammalian stage after the morula develops an eccentric cavity. Such terminology is a holdover from the heyday of the invertebrate and amphibian embryologists. In our laboratory the term "trophocyst" is used preferentially. The trophocyst of approximately 80 hours has differentiated no endoderm and consequentially possesses no blastocoel. By 90 hours a layer of endoderm has delaminated but is in direct contact with the inner cell mass from which it originated leaving no cavity between epiblast and hypoblast. Expansion of endoderm to line the peripheral trophoderm results in a true blastocoel between the epiblast and hypoblast, in this area, during the seventh day.

Huber (1915) stated that there was no Rauber's layer in the rat, but committed one glaring error which in turn biased his interpretations. He

turned the trophocyst upside down in his thinking, viewing the inner cell mass as a hypoblastic mass. He stated that the inner cell mass formed from an invagination of the epiblast (ventral trophoderm). This concept did not command much credence from later workers and was soon forgotten.

In the mouse the trophoderm over the inner cell mass becomes a flattened, single cell layer, "Rauber's layer", by 90 hours, and is distinctly present in all our mouse specimens from 90 hours to the sixth day when the ectoplacental cone is elaborated from it.

When the mouse trophocyst settles within a uterine crypt it typically makes contact peripherally approximately 100 to 150 μ from the bottom of the crypt. Cooksey (unpublished data) has found that Microtus ochrogaster typically settles to the bottom of the crypt. The trophodermal cells of the ventral hemisphere of the trophocyst swell somewhat as contact is made with the uterine epithelium during day four. They have not become "giant" but are comparatively larger than the elongate, flattened trophoderm cells of the dorsal hemisphere and Rauber's layer. By the latter part of the sixth day an occasional "giant" cell is present in the peripheral trophoderm, especially in the ventral trophoderm (Fig. 6). These cells, with nuclei up to 25 μ in diameter as compared to the average 15 μ of the surrounding decidua cell nuclei, bulge outward from the peripheral trophoderm into the surrounding endometrium. Those of the ventral trophoderm extend ventrally destroying the remainder of the crypt epithelium (Fig. 7).

By the end of the sixth day, when the ectoplacental cone is well formed and the epithelium lateral to the vesicle is destroyed (Fig. 4), there are no unusually large trophodermal cells. The relatively average sized trophodermal cells, from the ectoplacental cone to the ventral trophoderm, have completely

eliminated the adjacent crypt epithelium and begun the destruction of the endometrium. A few extremely large trophodermal cells present at the end of the sixth day could in no way be responsible for the even front of destruction of the endometrium.

Snell (1941) and Rugh (1964) stated that in the mouse, giant cells formed by the ectoplacental cone migrate ventrally along the outside of the peripheral trophoderm forming a loose band connected by protoplasmic strands to each other and Reichert's membrane spanning a channel network for maternal blood. In the specimens used for this study, no such migration of giant cells was observed. The enlarged trophodermal cells, the giant cells of the literature, develop at the point of contact of the trophoderm and uterine tissues, from the trophoderm involved. Snell (1941) and Rugh (1964) both stated that the primary role of the "giant" cells is that of attaching the trophocyst to the endometrium. This is doubtful, as the trophodermal cells do not attach to the endometrium but destroy and absorb it as they come in contact with it. As the embryonic vesicle increases in size, internal pressure would keep the trophoderm firmly pressed against the endometrium effectively anchoring it in position. The primary role of the trophodermal cells is that of elimination of the endometrium, facilitating the parturition of the embryonic vesicle at term.

In general the trophodermal cells, whether they are ectoplacental cone cells or peripheral trophoderm cells, do not become unusually large. An occasional giant cell can be found on the surface of the trophoderm next to the endometrium but these do not appear to be superior to the general trophoderm in their capacity for destruction of endometrium. There is no justification, in our mouse material, for an arbitrary division of "giant cells" into primary

or secondary giant cells because of size or order of appearance as was done by Snell (1941) and Rugh (1964).

Multinucleate cells, maternal in origin, are found within the decidua from about day six until the decidua is eliminated during the twelfth day. Their multinucleate condition is a result of degenerative changes taking place within the decidua. Binucleate cells predominate any field examined during the sixth and seventh days. Occasionally tetranucleate cells occur and rarely cells with eight nuclei are seen. After the seventh day the four and eight nuclei cells are more numerous.

Alden (1948) suggested that in the rat the ventral trophodermal cells form a primitive syncytium which moves along the basement membrane of the crypt epithelium lifting portions loose. In our material no evidence of a syncytium, primitive or otherwise, exists. The trophodermal cells of the ventral pole get quite large and pseudopodia extend ventrally from these cells destroying the uterine crypt epithelium but in all cases, distinct cell membranes limit each cell.

In the rat, Bridgman (1948) described a fetal plasmodium (the endovascular plasmodium of Duval, 1891) originating from the ectoplacental cone and growing outward from the cone along the sinuses within the endometrium. This fetal plasmodium reportedly destroys and replaces maternal endothelium within the mesometrial portion of the endometrium. Bridgman (1948) stated that the maternal endothelium becomes swollen prior to its removal by the fetal plasmodium. Cooksey (unpublished data) found a similar outgrowth in Microtus ochrogaster, but in none of the mouse material collected for this study was any evidence of a fetal plasmodium found invading maternal blood sinuses. The

maternal endothelium becomes swollen, especially close to the trophoderm, but maternal endothelium can be seen lining the sinuses right up to the edge of the cone tissue. The sinus lumens are continuous with the blood cavities within the ectoplacental cone which are bounded by embryonic cells.

This is not to say that the forming channels within the cone match exactly with existing sinuses within the endometrium. The channels form within the cone before there is maternal blood pressure to force them open. As the ectoplacental cone trophoderm destroys the adjacent endometrium, maternal capillary sinuses are ruptured. If a wall of cone tissue confronts the ruptured sinus, the leak is effectively blocked but if there is an opening into the channel network in apposition to the rupture, pressure of the blood would force open the ectoplacental cone vascular net. The same is true for the trophodermal network which surrounds the remainder of the embryonic vesicle. The channels are there within the trophoderm, potentially, and as the trophoderm destroys the endometrium, capillaries are breached allowing maternal blood to flow through the trophodermal channels.

The problem of the presence or absence of syncytial tissue in the placenta of the mouse is old and one not easily resolved. Kirby and Bradbury (1965) and Enders (1965) published quite convincing electron photomicrographs of the definitive placental barrier showing a three layered trophoderm, two layers of which are probably syncytial. But this is in the placenta of 15 days or older and leaves nine days from the appearance of the ectoplacental cone at day six and the 15 day stages examined by Enders. Jenkinson (1900) and Robinson (1904) referred to the ectoplacental cone as a trophoblastic syncytium. The ectoplacental cone maintains its identity as such only from day six to about day ten when chorionic villi begin to disrupt it. We found

only four examples of syncytial masses in 22 specimens examined. The four examples ranged from a syncytial cap on the ectoplacental cone to small balls of syncytial tissue within the cone or peripheral trophoderm. These were isolated and unrelated cases and no continuity was found in their appearance. We consider them abnormal, possibly resulting from poor fixation.

If there is any syncytial tissue in the mouse placenta from day 10 to day 15 it is in the labyrinth region where the trophodermal layer investing the invading chorionic villi is drawn extremely thin. With the light microscope, it is impossible to determine whether cell membranes remain within the trophoderm at these points. In the clumps and columns of tissue that surround the villous trees and form a layer covering the distal surface of the labyrinth, cell membranes are distinguishable at parturition. It is only within the labyrinth region that the possibility of syncytial tissue exists. Enders (1965) took his material from the labyrinth region of various placentas. Our observations seem to bear out Enders as far as he goes in his paper.

On the eighth day a mesodermal mass arises at the posterior end of the primitive streak, grows out across the extraembryonic coelom and makes contact with the serosa on the ninth day. This mesodermal mass connecting primitive streak to serosa is the body stalk. Probably no one structure has been more misunderstood in mouse and rat placentation than the body stalk. This structure has been consistently, down through the years, called the allantois. Snell (1941) referred to the body stalk as the "allantois" and in the next sentence mentioned its lack of an endodermal lined cavity. Rugh (1964), borrowing heavily from Snell, faithfully committed the same error, handing it down to future generations of workers. The same holds true for Everett (1935) and Bridgman (1948) in their papers on placentation in the rat.

The allantoic blood vessels push out through the relatively solid proximal portion of the body stalk and continue within the thin mesodermal wall of the bulbous distal portion. When the body stalk and the serosa fuse, during the ninth day, the serosa is vascularized by the allantoic vessels and becomes a true chorion.

The absence of allantoic involvement in the formation of the placenta precludes the existence of chorioallantoic villi as described by Mossman (1937) for the Muridae and Bridgman (1948) for the rat. In the mouse, Mus musculus, the villi are definitely chorionic, not chorioallantoic.

With the settling of the trophocyst into the uterine crypt the decidual reaction sweeps through the endometrium from crypt epithelium outward through all but a thin layer of stratum spongiosum. The cellular hypertrophy which accompanies the decidual reaction brings about an increase in tissue volume that is reflected in the uterine swellings. A second important result is that the endometrium dorsal to the embryonic vesicle swells and effectively seals off the uterine lumen in the area of the embryo. The uterine lumen remains open anterior and posterior to the swelling. The total length of uterine lumen blocked never exceeds 1.5 mm. With the continued growth of the embryonic vesicle the endometrium anterior and posterior to the vesicle is bulged out into the open uterine lumen between the swellings, overriding the floor or ventral surface of the uterine lumen. In longitudinal sections of uterine swellings, through day 11, the original uterine lumen may still be seen as narrow tongue-like clefts lying ventrally beneath the greatly enlarged embryonic swellings and are continuous with the uterine lumen between swellings. The distance between these tips of uterine lumen is approximately 1.4 mm on day ten. The peripheral trophoderm destroys the anterior and posterior

endometrium and then breaks down itself. Laterally and ventrally the trophoderm destroys the endometrium to within a few cells of the circular muscle before it degenerates. The decidual reaction progresses until only a thin band of stratum spongiosum remains. The trophoderm seemingly destroys these swollen decidual cells leaving the thin band of unaffected stratum spongiosum. The endometrium and peripheral trophoderm have been eliminated by the end of day 12 but Reichert's membrane and the distal endoderm are still intact separating the yolk sac vesicle from the uterine lumen. By the 14th day this last barrier ruptures and the embryo, surrounded by the yolk sac, is suspended within the uterine lumen.

With the destruction of the endometrium and the breakdown of the trophoderm, a narrow band of endometrium is left bare. Proliferation of the surrounding uterine epithelium completely covers this band by the 14th day.

The yolk sac has been suggested as a source of nutrition to the fetus in the rat (Everett, 1935; Bridgman, 1948) and in the mouse (Rugh, 1964). Everett stated that the yolk sac of rats is not secondary in importance to the allantoic vessels of the placenta. Blood from the decidual sinuses flows through the trophodermal channel network and comes in contact with Reichert's membrane. Nutrients pass through Reichert's membrane into the yolk sac cavity and are absorbed by the yolk sac epithelium. It is generally believed that the yolk sac does indeed aid in the securing of nutrients but doubt has been cast upon its importance by Noer and Mossman (1947). They tied off the allantoic blood vessels in one group of rats, in a second group they tied off the vitelline blood vessels and in a third group they tied off both sets of vessels. Those rats deprived of the allantoic vessels died within 60 to 170 minutes. No harm came to those that were deprived of the vitelline system only. The fetus

did die within four days following vitelline vessel ligation supposedly due to the passive constriction of the growing fetus by the dead, non-expanding yolk sac.

Bridgman (1948) described an active penetration of the placental pad by the yolk sac villi in the rat on the 15th day. There is no yolk sac involvement in the placental pad in the mouse. It is readily determined that the vessels within the villi are allantoic blood vessels and that the villi are chorionic villi. The diameter of the vesicle at the distal end where the yolk sac meets the cone increases from 70μ at day six to 3 or 4 mm near term. From day six to day nine this increase is due to expansion of the embryonic vesicle and subsequently from rapid proliferation of chorionic villi. Proliferation and increase in size of chorionic villi result in overgrowth at the junction and displacement of the yolk sac with investment of marginal chorionic villi with yolk sac epithelium for a short distance producing a superficial impression of "yolk sac villi" of previous authors.

The placenta of the mouse is functional as soon as the chorionic villi push into the maternal blood channels within the cone. This brings fetal and maternal blood into close proximity. But it is not until the 15th day that the placenta has reached its definitive state. By this time the peripheral endometrium is gone, Reichert's membrane has ruptured and the chorionic labyrinth is essentially complete. In the remaining four days the labyrinth region will increase in depth about 0.5 mm by growth of the chorionic villi.

It is impossible to determine the condition of the tissues between fetal and maternal blood with the light microscope. It is possible to detect the presence of trophoderm, affirming the definitive placenta of the mouse, Mus musculus, as hemo-chorial. Whether the trophodermal layer is syncytial or if

it is composed of several layers we can not say on the basis of light microscopy alone. There is no reason to doubt the validity of the work of Wislocki and Dempsey (1955) on the rat and Enders (1965) and Kirby and Bradbury (1965) on the mouse. Their works are in complete agreement on the three layered nature of the trophoderm. Enders (1965) included a statement by Mossman as to the possible origin of these various layers. He described them as (1) a layer of trophodermal giant cells in contact with the maternal blood spaces (2) a syncytial layer from the ectoplacental cone cells and (3) a layer from the serosal or chorionic ectoderm, probably syncytial.

It would be a great aid if a developmental study on placentation in the mouse would be done using the electron microscope. On the basis of our study, we must disagree with Mossman's interpretations. The classical giant cells of the literature were found in the peripheral trophoderm and covering the ectoplacental cone where they were in contact with the endometrium and aided in its destruction. In the term-placenta of the mouse, these large trophodermal cells are still present along the distal border of the placental pad in contact with maternal tissue. If one takes notice of this fact and notes, also, that Enders stated that trophoderm two is definitely syncytial and trophoderm one is probably syncytial, he can arrive at a different explanation of the origin of the tissues of the placental barrier.

Trophoderm t^1 , the layer adjacent to the fetal endothelium, originates from the chorionic ectoderm. The second trophodermal layer encountered is the lamina or solid base of the cone. As the chorionic villi lengthen, this laminar tissue invests each villus with the t^2 trophoderm layer. The third layer t^3 is derived from the ectoplacental cone cells which have remained bordering the maternal blood channels.

SUMMARY

Reproductive tracts of Swiss white mice were collected at intervals of six hours from ovulation to 11 days and 24 hours from 12 days to term.

The embryos enter the uterus at 80 hours post ovulation, become spaced equidistant along the length of the uterus and settle into crypts. The trophodermal cells, in contact with the uterus, swell and destroy the adjacent uterine epithelium.

During the sixth day, Rauber's layer proliferates, forming the ectoplacental cone which in turn destroys the adjacent maternal tissue dorsal to the embryo while the trophoderm destroys the maternal tissue lateral and ventral to the embryo. The peripheral trophoderm and ectoplacental cone form a mesh of channels which fills with maternal blood as maternal blood vessels are ruptured in their destruction.

The body stalk forces the serosa against the ventral surface of the cone during day nine. The allantoic blood vessels vascularize the serosa forming a chorion. Chorionic villi push into the base of the cone which gives way before the advance investing each villus with additional ectoderm. The villi push deeper into the cone interdigitating with the maternal blood channels bringing the two blood systems into close proximity.

By the end of the 12th day the trophoderm has destroyed the endometrium lateral and ventral to the embryonic vesicle and in turn destroys itself and the distal endoderm.

From day 15 through parturition little change occurs in the placenta. The definitive condition of the white mouse placenta is hemo-chorial.

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The author also wishes to accord recognition to his parents and grandmother for invaluable assistance during this study.

Explanation of Figures

Figure 1. Trophocyst, approximately 80 hours post ovulation, in the intramural portion of the oviduct. (210X)

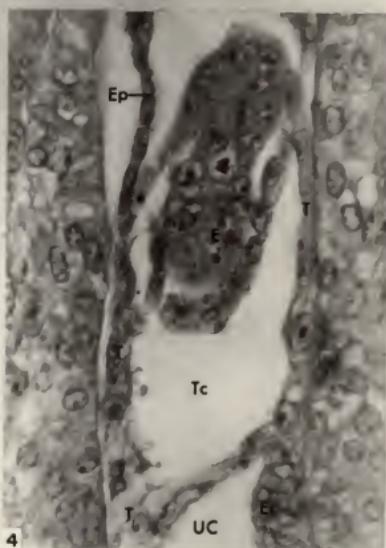
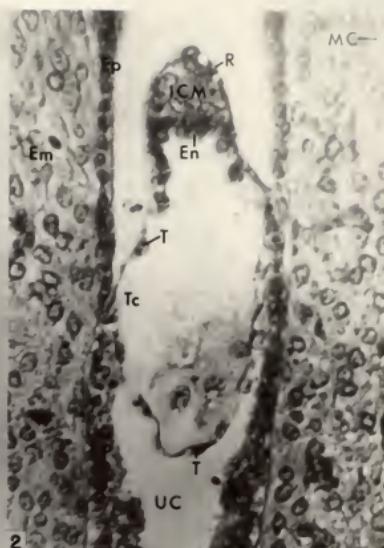
Figure 2. Trophocyst, approximately 90 hours post ovulation, settled in a uterine crypt. Destruction of epithelium begun. (350X)

Figure 3. Higher magnification, point of invasion of the uterine epithelium by a trophoderm cell. (700X)

Figure 4. Five day embryo within a uterine crypt. Destruction of uterine epithelium lateral to embryo completed. (580X)

Legend

E	Embryonic mass
Em	Endometrium
En	Endoderm
Ep	Uterine epithelium
ICM	Inner cell mass
L	Lumen of oviduct
MC	Maternal capillary
R	Rauber's layer
T	Trophoderm
TC	Trophocoel
UC	Uterine crypt



Explanation of Figures

Figure 5. Peripheral area from a day-6 embryo with peripheral blood channels formed between trophodermal cells, two of which have penetrated the mucosa. The outer layer of trophoderm progressively destroys and ingests endometrial cells. (520X)

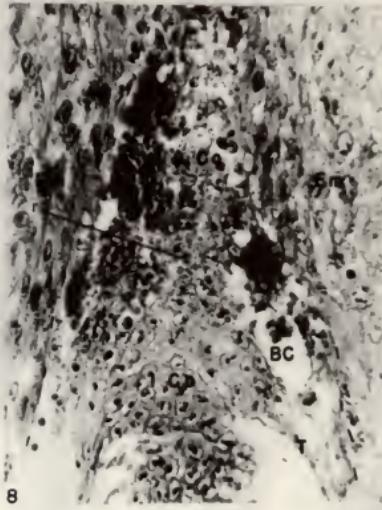
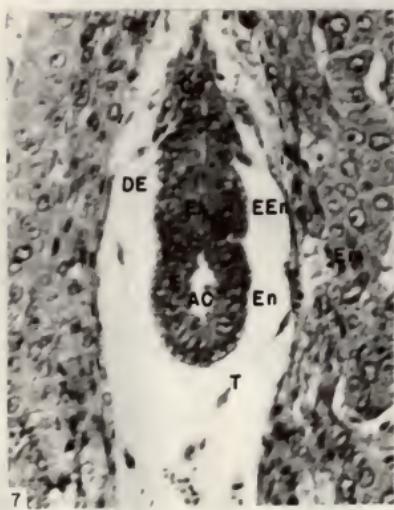
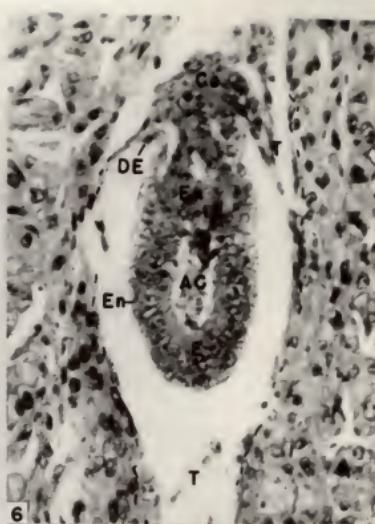
Figure 6. Early day-6 embryo showing the beginning of the formation of the proamniotic cavity by dissolution of central cells of the embryonic mass. (320X)

Figure 7. Longitudinal section of an embryo six days post ovulation. The proamniotic cavity contains only remnants of cellular debris and an equatorial constriction has formed a division between embryonic and extraembryonic portions of the mass. The ectoplacental cone, formed from Rauber's layer, is continuous with the peripheral trophoderm. (300X)

Figure 8. Detail of the ectoplacental cone of an early 7-day embryo. Intercellular channels, containing maternal blood, have formed within the cone and connected with surrounding maternal blood vessels. (410X)

Legend

AC	Proamniotic cavity
ACo	Apical portion of the cone
BC	Maternal blood channels
C	Yolk cavity
Co	Ectoplacental cone
DE	Distal endoderm
E	Embryonic ectoderm
Eb	Embryo
EE	Extraembryonic Endoderm
En	Endometrium
En	Embryonic endoderm
Ex	Extraembryonic ectoderm
I	Maternal-embryonic interface
ICo	Inner portion of the cone
r	Remnant of endometrial cells
T	Trophoderm



Explanation of Figures

Figure 9. Seven day embryo prior to formation of the amnion. The proamniotic cavity is continuous with the cavity in extra-embryonic ectoderm. (170X)

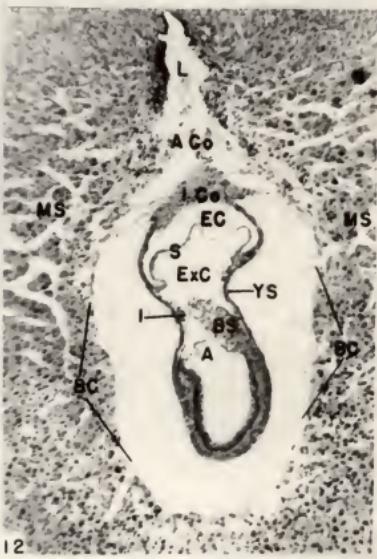
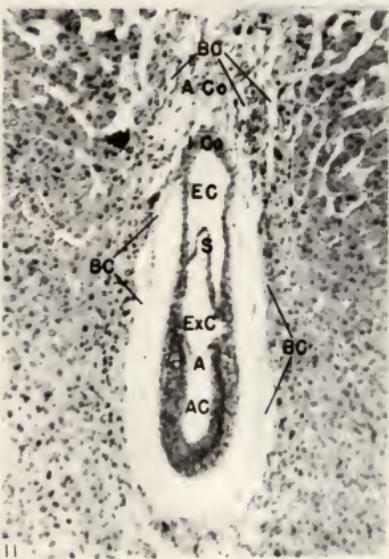
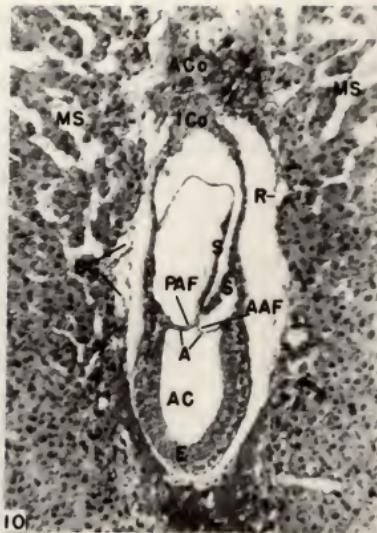
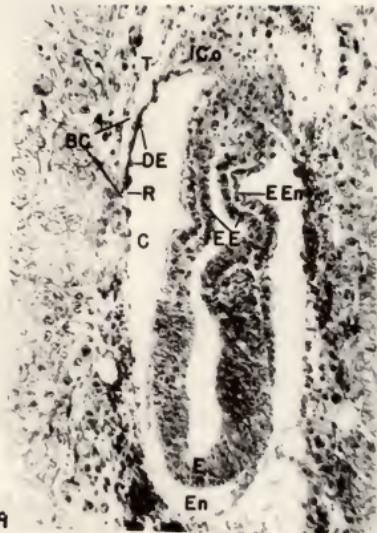
Figure 10. Longitudinal section of a day-7 embryo with anterior and posterior amniotic folds. The greatly enlarged maternal capillary sinuses are evident in the dorsal half of the endometrium and to a less extent laterally. (90X)

Figure 11. Early day-8 embryo with amnion and serosa differentiated. Blood channels in the apical portion of the cone are continuous with the maternal sinuses in the endometrium. (90X)

Figure 12. Late day-8 embryo. The body stalk has begun expansion across the exocoel. Shrinkage of the specimen resulted in unusually large spaces in the apical cone. (60X)

Legend

A	Amnion
AAF	Anterior amniotic fold
ACo	Apical portion of the cone
AC	Amniotic cavity
BC	Blood channels
BS	Body stalk
C	Yolk cavity
DE	Distal endoderm
E	Embryonic ectoderm
EC	Ectoplacental cavity
EE	Extraembryonic ectoderm
EEn	Extraembryonic endoderm
En	Embryonic endoderm
ExC	Extraembryonic coelom (exocoel)
ICo	Inner portion of the cone
L	Uterine lumen
MS	Maternal sinus
PAF	Posterior amniotic fold
R	Rauber's layer
S	Serosa
T	Trophoderm



Explanation of Figures

Figure 13. Transverse section through an early day-9 embryo posterior to the amnion. The body stalk has made first contact with the serosa which has collapsed against the cone, almost obliterating the ectoplacental cavity. (115X)

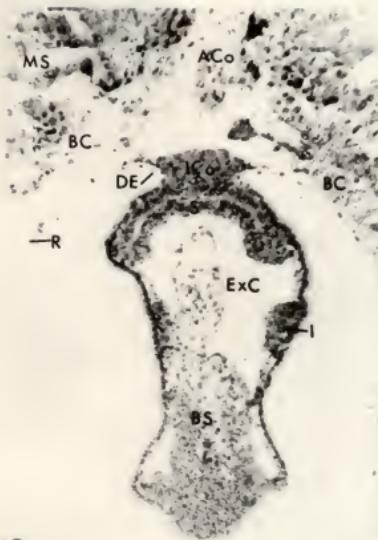
Figure 14. Section through the ectoplacental cone of an embryo about 12 hours older than the one shown in Fig. 13. The body stalk has made contact with the entire ventral surface of the cone. The columnar serosal ectoderm has fused with the base of the cone, completely eliminating the ectoplacental cavity. (95X)

Figure 15. Section through the cone of a slightly older embryo, late day-9. Beginning chorionic villi each contain an allantoic blood vessel. (110X)

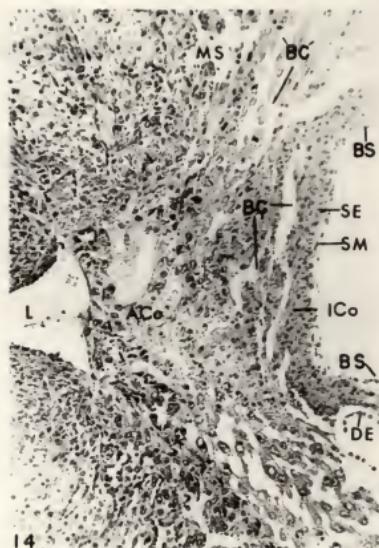
Figure 16. Section of the cone-chorionic area of an early day-10 embryo. Short chorionic villi, 40 to 60 μ in length, have extended toward the blood channels. (110X)

Legend

A	Amnion
ABo	Apical portion of the cone
BC	Blood channels
BS	Body stalk
CV	Chorionic villi
DE	Distal endoderm
EC	Ectoplacental cavity
ExC	Extraembryonic coelom (exocoel)
Em	Endometrium
I	Blood islands
ICo	Inner portion of the cone
L	Uterine lumen
MS	Maternal sinus
R	Reichert's membrane
S	Serosa
SE	Serosal ectoderm
SM	Fused serosal and body stalk mesoderm
YS	Yolk sac
--	Dashed line denotes embryonic-maternal interface



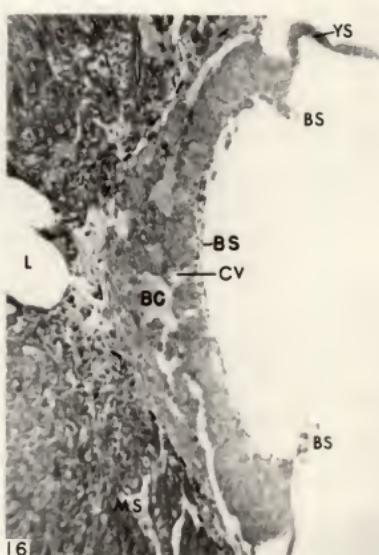
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Explanation of Figures

Figure 17. Developing placental pad of a ten day embryo. Blood channels within old cone area, now labyrinthine area, having expanded and are obviously continuous with the surrounding maternal sinuses. The uterine lumen (L), yet visible at this stage, becomes obliterated within the next day. (20X)

Figure 18. Developing placental pad of an eleven day embryo. The decidual reaction has progressed to within a few cells of the circular muscle and chorionic villi have filled the cone area leaving only a thin lamina (La) adjoining the maternal layer. The metrial gland has developed within the circular muscle layer, medially over the pad. (20X)

Figure 19. Placental pad of a twelve day embryo. The lamina, with its covering of trophodermal "giant cells", has progressed well into the endometrial layer, and the labyrinthine area expanded comparably. Only a thin band of endometrium remains. (10X)

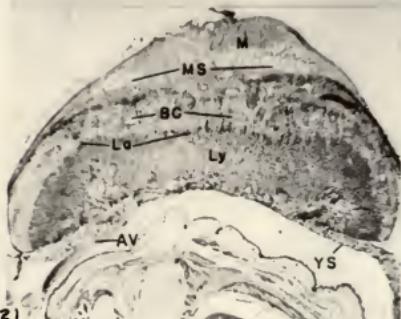
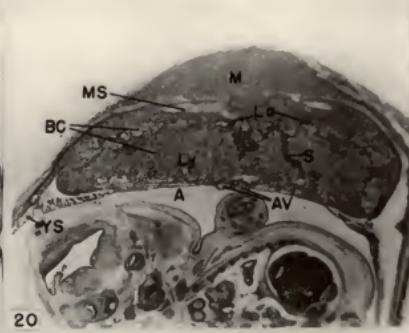
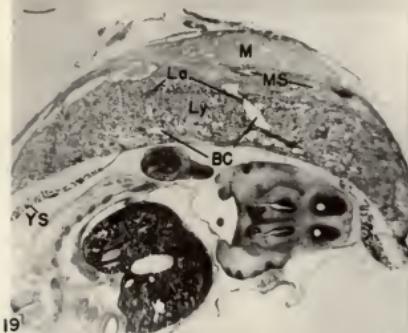
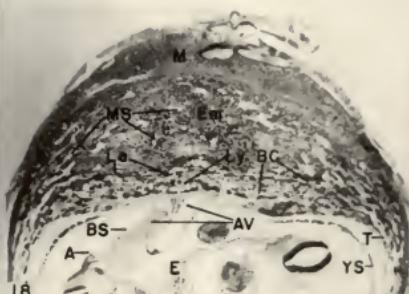
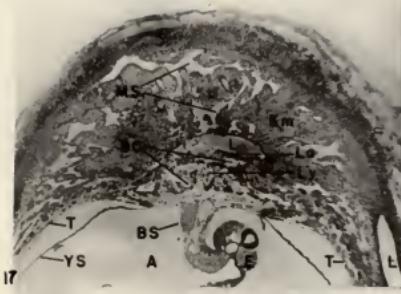
Figure 20. Placental pad of a 15-day embryo. The placenta is essentially complete at this stage and the metrial gland has developed maximally. Laminar septa (S) remain within the labyrinthine region, between expanding chorionic villi. (10X)

Figure 21. Placental pad of a 17-day embryo. Endometrium with maternal sinuses has been reduced to a minimal layer over the pad, adjoined by a trophodermal meshwork containing blood channels (BC) but not yet invaded by chorionic villi. The metrial gland has failed to keep pace with the developing placenta, and actually regresses after day 16. (10X)

Figure 22. Term placenta from the distal swelling of a uterine horn of a mouse killed during parturition. The non-villous layer containing blood channels, outside the lamina has been reduced to near-disappearance in some places, and the lamina remains only as a thin limiting layer outside the labyrinth. (10X)

Legend

A	Amnion
AV	Allantoic blood vessels
BC	Blood channels
BS	Body stalk
E	Embryo
Em	Endometrium
L	Uterine lumen
La	Laminar region
Ly	Labyrinth
M	Metrial gland
MS	Maternal blood sinuses
S	Laminar septa
T	Trophoderm
YS	Yolk sac
--	Dashed line denotes embryonic-maternal interface



Explanation of Figures

Figure 23. Longitudinal section through a ten day uterine swelling. The thickened endometrium ventral to the embryonic vesicle is indicative of the original site of attachment. (15X)

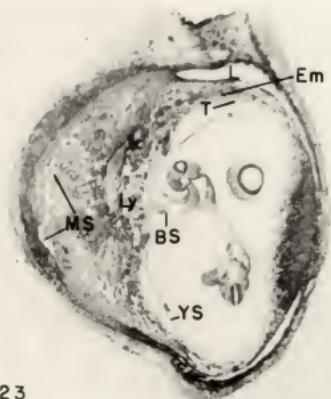
Figure 24. A transverse section of an 11-day uterine swelling through the original attachment site. (15X)

Figure 25. Detail of the margin of the placental pad of a 12-day embryo showing the remnant of the peripheral endometrium. Reichert's membrane is still intact surrounding the embryonic vesicle. (55X)

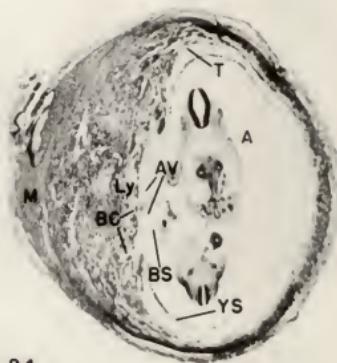
Figure 26. Detail of the margin of the placental pad of a 15-day embryo. Ventral and lateral growth of the placental pad has formed a groove in Reichert's membrane around the periphery of the pad. Reichert's membrane has ruptured around the yolk sac and contracted toward the endometrial remnant. (80X)

Legend

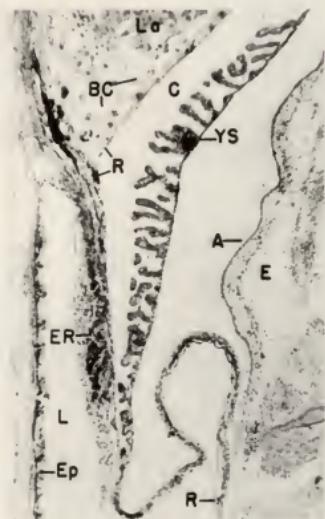
A	Amnion
AV	Allantoic blood vessels
BC	Blood channels
BS	Body stalk
C	Yolk cavity
E	Embryo
Em	Endometrium
Ep	Uterine epithelium
ER	Endometrial remnant
L	Uterine lumen
La	Lamina
Ly	Labyrinth
M	Metrial gland
MS	Maternal sinus
P	Placental pad
R	Reichert's membrane
T	Trophoderm
YS	Yolk sac



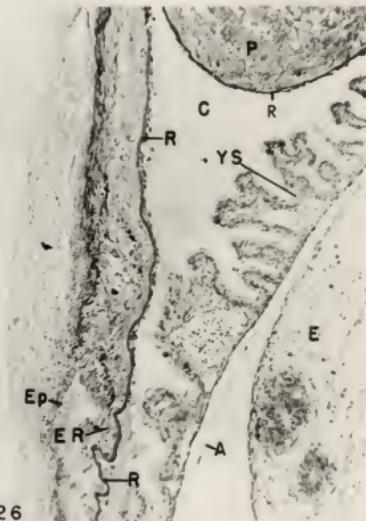
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Explanation of Figures

Figure 27. Section through the center of a placental pad of a day -9 embryo to show early villous formation. The serosal ectoderm has assumed a columnar appearance. Allantoic blood vessels fill the irregularities (villous evaginations) in the ventral surface of the serosa. (140X)

Figure 28. Placenta of a ten day embryo. Chorionic villi have expanded to 40 - 60 μ in length and pushed into and across the basal layer of blood channels. (110X)

Figure 29. Section of an eleven day placenta. Chorionic villi have increased in length to about 250 μ and have branched. The nucleated embryonic blood cells serve as specific markers for allantoic blood vessels and villous ramifications. (100X)

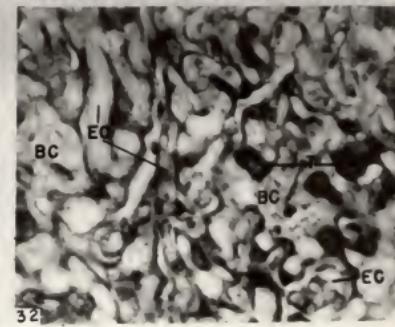
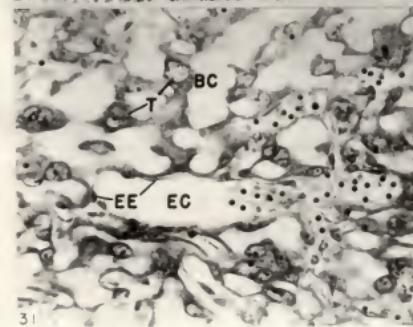
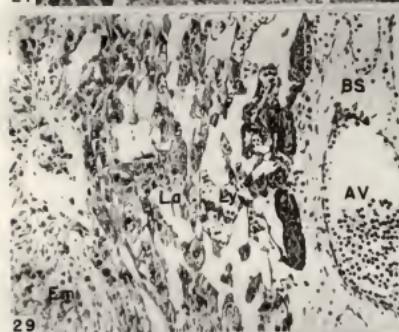
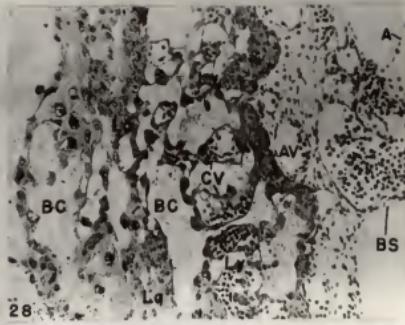
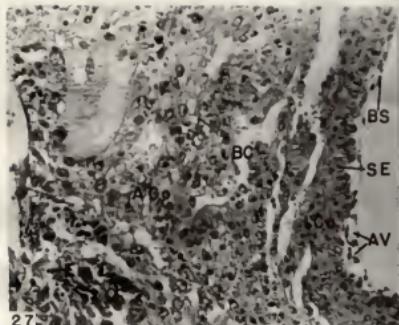
Figure 30. Section of a twelve day placenta showing a main villous trunk with highly branched villi in the labyrinth region. Villi extend to the lamina. (100X)

Figure 31. Section of a fifteen day placenta, showing an embryonic capillary separated from maternal blood by embryonic endothelium and trophoderm. Embryonic blood cells are mostly nucleated. (600X)

Figure 32. Detail of a term placenta. Embryonic blood, no longer nucleated, is separated from maternal blood by embryonic endothelium and trophoderm. (600X)

Legend

A	Amnion
ACo	Apical portion of the cone
AV	Allantoic blood vessels
BC	Blood channels
BS	Body stalk
CV	Chorionic villus
EC	Embryonic capillary
EE	Embryonic endothelium
Em	Endothelium
ICo	Inner portion of the cone
La	Lamina
Ly	Labyrinth
T	Trophoderm



Explanation of Figures

Figure 33. Uterus of a nine day pregnant mouse. The vascular supply to each uterine swelling from the uterine artery is shown. Magnification is shown by the mm rule.

Figure 34. Uterus of a twelve day pregnant mouse.

Figure 35. Dissection of a swelling in a twelve day pregnant uterus. The embryo is evident with its left side against the developing placental pad.

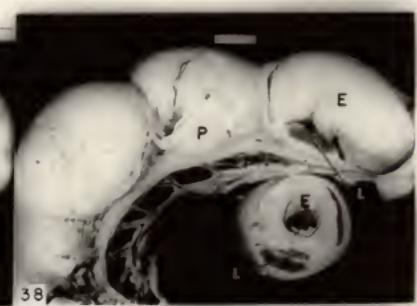
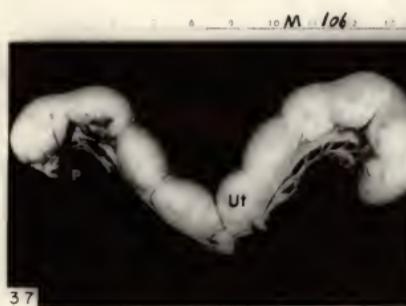
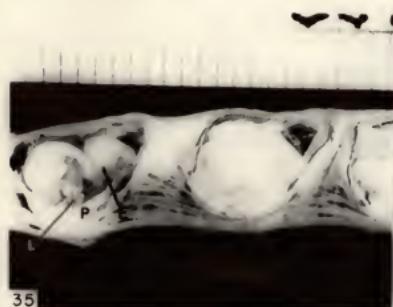
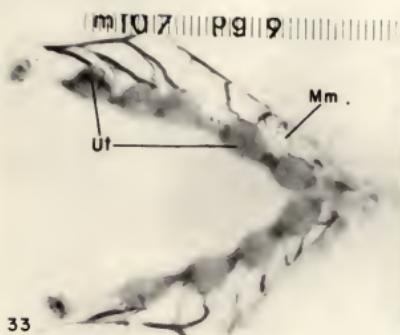
Figure 36. Uterus of a fifteen day pregnant mouse.

Figure 37. Uterus of an eighteen day pregnant mouse.

Figure 38. Dissection of four eighteen day swellings. The first swelling on the left has just the muscularis removed. The second has the yolk sac vesicle dissected off. The vitelline vessels are evident lying across the fetus. The remaining two swellings are a longitudinal section and a transverse section through the placental pad.

Legend

E	Embryo
L	Labyrinthine region of placenta
Mm	Mesometrium
P	Placental pad
Ut	Uterine swelling



PLACENTATION IN THE LABORATORY MOUSE

by

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B. S., Baker University, 1963

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Prior to this study, knowledge of placentation in the white mouse was limited to general information such as was available in Mossman's 1937 classic work and from homologies drawn between rat and mouse placentation. Fifty-two sets of closely staged mouse embryos were collected and studied in an attempt to provide direct information on placentation in the mouse.

The embryos enter the uterus during the fourth day and settle into crypts off the uterine lumen. During days four and five the trophoblast destroys and removes the uterine epithelium lateral to the embryonic vesicle.

During the sixth day the inner cell mass increases greatly, forming the embryonic mass and Rauber's layer proliferates forming the ectoplacental cone. Intercellular vesiculation results in an interconnected network of channels within the apical half of the ectoplacental cone by the end of the day.

The extraembryonic membranes form during the seventh day. Reichert's membrane appears between the distal endoderm and the peripheral trophoderm. The peripheral trophoderm destroys an eight to ten cell layer of endometrium, reaching and rupturing the maternal capillary sinuses allowing maternal blood to flow through the loosely associated trophodermal cell layer. A similar occurrence, with the ectoplacental cone destroying the endometrium dorsal to the vesicle, vascularizes the cone blood channels.

The body stalk arises from the posterior end of the embryo, during day eight, and grows into the exocoel toward the base of the ectoplacental cone. The body stalk contacts the serosa on day nine forcing it dorsally against the base of the cone. Irregular evaginations appear along the ventral surface of the serosa, each containing an allantoic capillary. By the end of the

tenth day the allantoic capillaries have pushed into the base of the cone as much as 250 μ each invested with a serosal and laminar ectoderm covering. The chorionic villi push into and cross the maternal blood channels increasing the ectodermal investment of the chorionic villi with cone cells from the maternal blood channels.

Lateral growth of the placenta increases rapidly, altering its shape from conical to discoid. Chorionic villi continue their growth, increasing the labyrinth from 300 μ by 3 mm on day 11 to 1 by 6 mm on day 14. The mesometrial endometrium is reduced from 1.5 mm to 200 μ during this time.

Peripherally, the trophoderm destroys and eliminates the lateral and ventral endometrium and in turn destroys itself by the end of day 12. Reichenert's membrane and the distal endoderm rupture on the 14th day, freeing the embryonic vesicle into the uterine lumen.

The placenta is complete on the 15th day, the labyrinth increases another 0.5 mm in thickness by parturition on day 19. The lamina and the trophodermal maternal blood channel network are still present to some degree at parturition. The trophoderm remains between the fetal capillary endothelium and the maternal blood. The definitive placenta of the laboratory mouse is hemo-chorial.

With the completion of our study we found that, while some similarities exist between placentation in the mouse and that reported for the rat, there are decidedly great differences.

The invasion of maternal tissue by embryonic is cellular. There is no evidence of a syncytiotrophodermal invasion followed by a cytotrophodermal invasion. In the white mouse the embryonic cells destroy the maternal tissue and phagocytize the debris. There is no yolk sac involvement in the placenta

disc of the white mouse. There is no endovascular plasmodium as reported for the rat and Microtus ochrogaster.

The allantois develops relatively late in the mouse, after the placenta is well along in its development. The villi are chorionic, not chorioallantoic as inferred in the literature.